

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: 04.09.85

(21) Application number: 81303585.4

(22) Date of filing: 05.08.81

(51) Int. Cl.⁴: **C 07 K 7/06, C 07 C 101/30, C 07 C 101/34, A 61 K 37/02 // C07C101/04, C07C101/24**

(64) **Enzyme inhibitors.**

(39) Priority: 06.08.80 GB 8025579
12.06.81 GB 8118194

(43) Date of publication of application:
10.02.82 Bulletin 82/06

(45) Publication of the grant of the patent:
04.09.85 Bulletin 85/36

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(56) References cited:
DE-A-2 714 141
DE-A-2 811 267
US-A-4 198 398

Chemical Abstracts vol. 75, no. 23, December 1971 Columbus, Ohio, USA K. SHIGEZANE et al. "Synthesis of antirenin active peptides. II." page 350, abstract no. 141149r

Chemical Abstracts vol. 85, no. 17 October 1976 Columbus, Ohio, USA M.J. PARRY et al. "Bio.isosteres of a peptide renin inhibitor" page 65, abstract no. 117050n

(73) Proprietor: Aktiebolaget Hässle
Kärragatan 5
S-431 83 Mölndal (SE)

(72) Inventor: Szelke, Michael
10 North Drive
Ruislip Middlesex (GB)
Inventor: Jones, David Michael
89 Woodrow Avenue
Hayes Middlesex (GB)
Inventor: Hallett, Alan
73a Elm Road
Wembley Middlesex (GB)

(74) Representative: Näsman, Rune B. G. et al
AB Astra Patent and Trade Mark Department
S-151 85 Södertälje (SE)

(58) References cited:
Chemical Abstracts vol. 80, no. 1, January 1974 Columbus, Ohio, USA I. PARIKH et al. "Substrate analog competitive inhibitors of human renin" page 108, abstract no. 1086w
Chemical Abstracts vol. 87, no. 8, July 1977 Columbus, Ohio, USA K. POULSEN et al. "Competitive inhibitors of renin. A review" page 139, abstract no. 1561s

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European patent convention).

which is the tetrapeptide (II) modified at the Leu-Leu link, leucine of course being



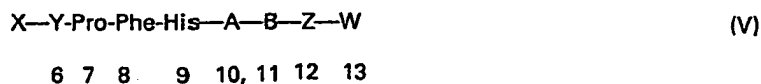
This analogue (III) was the first effective in-vivo inhibitor of renin and was shown to have significant anti-hypertensive action in Goldblatt hypertensive rats (Parry, Russell and Szelke p. 541 in "Chemistry and Biology of Peptides" Ed. Meienhofer, Ann Arbor Science Publishers 1972). Little or no attention has however been paid to the work, which the authors themselves were unable to pursue, in spite of considerable activity in the general field of substrate-based inhibitors for renin, reviewed for example by Haber & Burton, Federation Proc. 38 No. 13 2768-2773 (1979).

The Invention

The present invention is a development of the above work. Behind it is a concept of modifying peptide structures related to the peptide sequence at the site of action of renin on the natural substrate, by isosteric substitution at, at least, the site of cleavage. Optionally further there is isosteric substitution or other modification at other positions to increase stability or to modify the properties of the final peptide, for example its solubility under physiological conditions or its resistance to in vivo exopeptidase attack. Such modification may for example be by incorporation of residues other than those of the natural L-amino acids; by protection of the N-terminus with acetyl, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl or other groups; or by conversion of the C-terminal carboxyl to another functional group, e.g. the corresponding alcohol, present as such or in ether or ester form.

General reference to amino acids and amino acyl residues and side chains in both the description and claims herein is to be taken as reference to such whether naturally occurring in proteins or not and to both D- and L-forms, and amino is to be taken as including imino except where an aromatic acid, residue or side chain is specified.

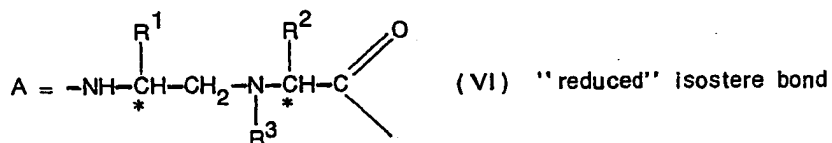
The compounds of the present invention, showing desirable renin inhibitory action, are of the general formula:



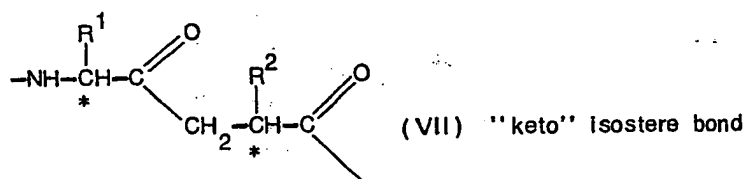
where Pro, Phe and His may be in substituted form;

X = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl or lower alkyl (primarily C₁-C₅); or an L- or D-amino- acyl residue, which may itself be N-protected similarly;

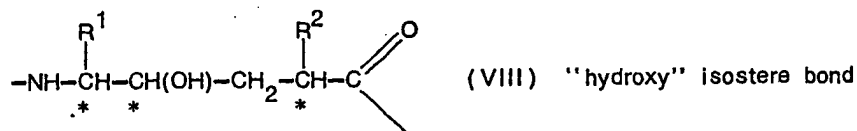
Y = D- or L-His or other D- or L- basic or aromatic amino-acyl residue, or is absent;



or



or



where R_3 is as set out above. The alternative isosteric substitutions set out herein may however be used.

Protective or substituent groupings as mentioned above may be any of these known in the polypeptide art amply disclosed in the literature and not requiring discussion at length here. Generally the selection of the 'protective' groups is according to their function, some being primarily intended to protect against undesired reaction during synthetic procedures while the N- and C-terminal substituents are for example directed against the attack of exopeptidases on the final compounds or to increase their solubility and hence physiological acceptability.

It is in particular possible for one or more remaining peptide bonds in the compounds of formula (V), (VA) or (VB) to be N-substituted with protective groups.

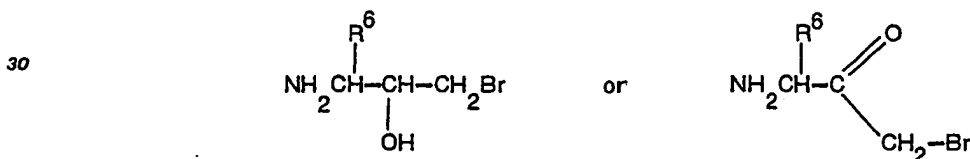
The invention further lies in the analogues for use as medicaments and particularly

i) in a diagnostic test for high renin states, blood pressure falling most when renin is high, or in a surgical prognostic test for reno-vascular hypertension (renal artery stenosis), by administration of said analogue followed by monitoring of blood pressure, and

ii) in the long and short term treatment of heart failure and all forms of hypertension particularly those associated with high serum renin levels, by administration of a renin-inhibiting amount of said analogue.

The long and short term response of blood pressure to renin inhibitors is predictive of surgical outcome. In all cases single and repeated doses and any conventional form of pharmaceutical composition may be used, for administration by intranasal or oral route, injection, or any other means as convenient. Amounts may for example be 0.001 to 10 mg/kg body weight daily more usually 0.01 to 1 mg, according to the potency of the analogue and the severity of the condition. Dosage unit compositions may contain such amounts or submultiples thereof to make up the daily dose. (Dosages herein and in the claims are related to the free base content where compounds are in salt form)

In production of the analogues use may be made of a method of making a hydroxy or keto isostere of a dipeptide wherein a derivative of a halohydrin preferably a bromohydrin or haloketone preferably a bromoketone



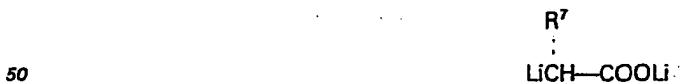
wherein R^6 is an amino acid side chain and the NH_2 and OH groups are in protected form is subjected to an alkylation procedure to attach a group



and gives the desired isostere as such or in protected form, R^7 being the same or a different amino acid side chain.

In particular the alkylation procedure may be

i) by reaction with an alkali metal carboxylic acid derivative preferably a lithium derivative



where R^7 is as above.

ii) by reaction with an alkali metal malonic ester derivative preferably a sodium derivative



where R^8 is an esterifying group and a halide preferably an iodide

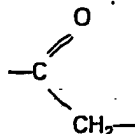


where R^7 is as above to give intermediate

Further analogues within formula (VA) are given in the present disclosure in Examples VI to IX, XI and XII. Analogues within formula (VB) are given in Examples V and X.

Synthetic Methods

- 5 The inventors have developed synthetic methods for the isosteric replacement of the peptide bond —CO—NH— with alternative groups, specifically $\text{—CH}_2\text{—NH—}$ (reduced), $\text{—CH}_2\text{CH}_2\text{—}$ (hydrocarbon),

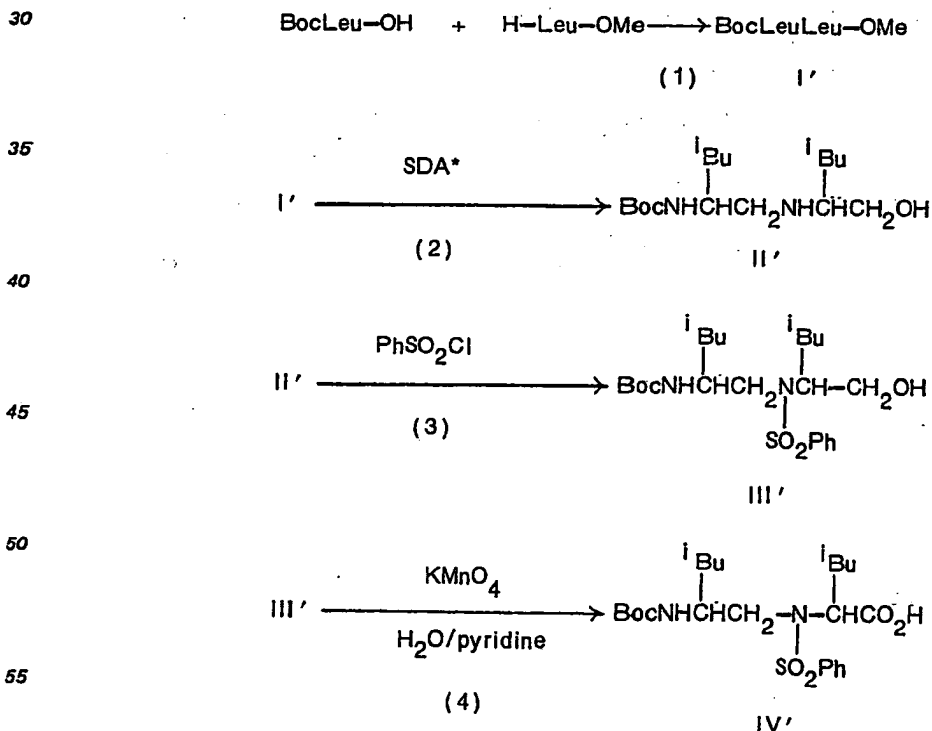


- 10 (keto) and $\text{—CH(OH)—CH}_2\text{—}$ (hydroxy) isosteres (see, e.g. Szelke, et al, pp. 57—70 in "Molecular Endocrinology" Vol. 1, Editors: MacIntyre and Szelke, Elsevier, Amsterdam 1977, and Hudson, Sharpe and Szelke, U.S. Patent 4 198 398 "Enkephalin Analogues").

- 15 Reference may be made to these publications for general discussion of such isosteric replacement. A reaction sequence for the preparation in particular of the reduced isostere of leucyl leucine for incorporation in the analogues disclosed herein is however for example:

SCHEME 1

Synthesis of the protected reduced isostere of L-leucyl-L-leucine



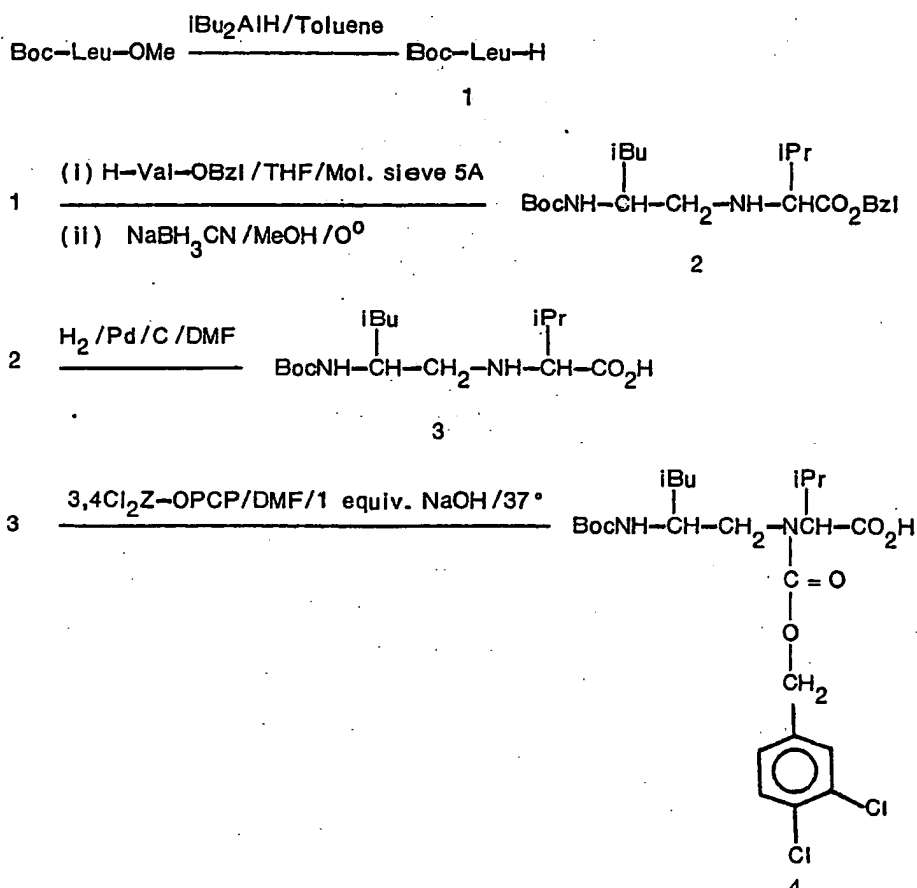
* Sodium di(methoxyethoxy) aluminium hydride

(1) Boc-Leucyl-leucine methyl ester

- 20 The dipeptide I' was prepared from Boc-leucine.H₂O (27.5 g, 0.11 mole) and leucine methyl ester.HCl (20 g, 0.11 mole) by mixed anhydride coupling using N-methyl morpholine and isobutylchloroformate. After a standard work-up procedure the dipeptide I' was obtained as white needles, 35.0 g (88%) from
25 EtOAc/petrol bpt 40—60°, m.p. 132—133°

SCHEME 3

Synthesis of the protected reduced isostere of L-leucyl-L-leucine



(1) Boc-L-Leucinal, 1

Boc-L-Leucine methyl ester (22.7 g, 90 mmoles) in dry toluene (250 ml) under N_2 was cooled to -78° and 25% di-isobutylaluminium hydride in toluene (130 ml, 225 mmoles) were added over 25 mins. keeping the temperature under -70° . The mixture was stirred for 15 mins. at -78° after completion of the addition, then MeOH (10 ml) was added cautiously. When effervescence ceased the mixture was poured into an ice-cold solution of Rochelle salt (100 ml of saturated solution + 600 ml H_2O). This mixture was shaken until an extractable solution was obtained. The toluene was separated and the aqueous phase re-extracted with ether (2 x 300 ml). Toluene and ether extracts were combined and dried (Na_2SO_4). The resulting oil was passed rapidly through a pad of silica gel in 15% EtOAc/petrol 40–60°. The crude aldehyde was obtained as an oil, weight 18.68 g. Nmr showed aldehyde content to be 85%, therefore yield of aldehyde: 15.9 g (83%).

Nmr (CDCl_3), τ : 0.45 (1H, s, CHO); 4.87 (H, br. d., Boc NH); 5.83 (1H, br. m., NH–CHCHO); 8.43–8.93 (12H, m, $(\text{CH}_3)_3\text{C}$, $(\text{CH}_3)_2\text{CHCH}_2$); 9.0 and 9.1 (12H, 2 x d, $(\text{CH}_3)_2\text{CH}$)
 TLC: (solvent 30% EtOAc/petrol 60–80°), R_f = 0.43.

(2) Boc-L-Leucyl-L-valine benzyl ester reduced isostere, 2

L-Valine-OBzl (10 mmoles, from EtOAc/1N NaHCO_3 partition of 3.8 g of p-toluene sulphonate salt) and Boc-L-Leucinal (2.54 g, 10 mmole aldehyde content) in dry tetrahydrofuran (20 ml) stood over 5Å molecular sieve (10 g) overnight. Sodium cyanoborohydride (630 mg, 10 mmoles) in MeOH (3 ml) was added with cooling, then left at room temperature for 30 mins. The mixture was diluted with methylene chloride (100 ml), filtered and evaporated to dryness. The residue was passed down a silica column in 20% EtOAc/petrol (60–80°) to remove polar impurities. Isostere containing fractions were combined. Crystallisation from petrol 60–80° at -20° gave large clusters of needles, 1.52 g (38%) m.p.

HOBt (0.92 g, 6 mmol) for 14½ hours* followed by acetylation for 1 hour.

The resin was then washed with DMF (3X), CH₂Cl₂ (3X) iPrOH (2X), CH₂Cl₂ (3X) and finally MeOH (3X) and dried to give 3.5353 g of product.

1.2 g of this material was treated with HF at 0° for 1½ hours in the presence of anisol (1.5 ml) then dried overnight over potassium hydroxide. The resin was then washed with DMF/water (1:1), acetic acid and finally acetic acid/water (1:1) to remove the peptide. These washes were combined and evaporated *in vacuo*.

The residue was dissolved in DMF (15 ml) and water (6 ml) thioethanol (5 ml) added and the pH of the solution brought to 8.0 with sodium carbonate. The reaction was stirred overnight the solvent evaporated and the residue applied to a Sephadex G 25 column (72 × 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 27—46 were combined and the solvent evaporated *in vacuo* and dried. Then 90% of the residue was taken (for the rest see Example III) and dissolved in anhydrous ammonia (100 ml) and small portions of sodium wire added until a permanent blue colour was achieved for 15 seconds. The ammonia was allowed to evaporate and the residue dried.

The residue was applied to a Sephadex SPC25 column (77 × 1.6 cms) eluted with 30% acetic acid at 40 mls/hr, with a sodium chloride gradient from 0.01M to 1M over two days collecting 6.6 ml fractions.

The product was contained in fractions 100—104. These were pooled, evaporated and the residue dissolved in glacial acetic acid and filtered to remove the sodium chloride. The solution was evaporated and desalted on a Sephadex G25 column (72 × 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 32—6 were pooled, evaporated, transferred to a vial and lyophilised.

Yield 13.4 mg

Product C₅₂H₇₄O₉N₁₂ MW. 1011.25

T.l.c. (silica) Rf 0.15 EtOAc/Pyr/AcOH/H₂O 40:20:6:11

Rf 0.40 nBuOH/Pyr/AcOH/H₂O 30:20:6:24

T.l.e. pH 2.1 1000V 30 min mobility 8.3 cm.

pH 6.5 1000V 30 min mobility 7.5 cm.

AAA 6N HCl + phenol 110°, 40 hours, peptide content 72%

His: 1.97; Pro: 1.01; Val: 1.02; Tyr: 0.98; Phe: 1.01.

Example II

H—Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH (H—79)

Fractions 80—84 of the SPC 25 Sephadex Column from the previous synthesis were combined, evaporated and the residue dissolved in glacial acetic acid and filtered to remove sodium chloride. The solution was evaporated and the product desalted on a Sephadex G25 column (72 × 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 32—9 were pooled, evaporated, transferred to a vial and lyophilised.

Yield 23.6 mg

Product C₄₆H₆₇O₈N₉ MW 874.10

T.l.c. (silica) Rf 0.29 EtOAc/Pyr/AcOH/H₂O 40:20:6:11

Rf 0.46 nBuOH/Pyr/AcOH/H₂O 30:20:6:24

T.l.e. pH 2.1 1000V 30 min mobility 7.5 cms

pH 6.5 1000V 30 min mobility 8.3 cms

AAA 6N HCl + phenol, 110°, 40 hours, peptide content 85%

His: 0.97; Pro: 1.08; Val: 0.99; Tyr: 0.97; Phe: 1.00.

The above example illustrates how Y in formulae (V), (VA) and (VB) may be absent.

Example III

H-His-Pro-Phe-His-Leu-reduced (SO₂Ph)-Leu-Val-Tyr-OH (H—78)

In the synthesis of compound H76 10% of the residue from the Sephadex G25 column after the HF and thioethanol treatments of the resin was kept.

This material was applied to a Sephadex SPC25 column (77 × 1.6 cm) eluted with 30% acetic acid at 20 mls/hr with a sodium chloride gradient from 0.01M to 1M over 2 days collecting 6.6 ml fractions.

The product was contained in fractions 74—7. These were pooled, evaporated, dissolved in glacial acetic acid and filtered to remove sodium chloride. The solution was then evaporated and desalted on a Sephadex G25 column (72 × 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 31—4 were pooled evaporated, the residue transferred to a vial and lyophilised.

Yield 0.6 mg

Product C₅₈H₇₈O₁₁N₁₂S MW: 1151.40

T.l.c. (silica) Rf 0.31 EtOAc/Pyr/AcOH/H₂O 40:20:6:11

T.l.e. pH 2.1 1000v 30 min mobility 5.4 cms.

AAA 6N HCl + phenol, 40 hrs, 110°, peptide content 64%

His: 1.93; Pro: 1.08; Val: 1.05; Tyr: 0.96; Phe: 0.97.

Coupling to Resin Ester

Boc-His(DNP)-O-Resin (2.5g 0.55 mmol) was deprotected with 50% TFA/CH₂Cl₂ and Boc-Ile-OH (0.748g 3mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6.0mmol) for 2 hours, then acetylated with acetyl imidazole (0.55g, 5mmol) overnight.

- 5 After deprotection with 40% TFA/CH₂Cl₂, Boc-Leu-reduced (3,4-Cl₂-Z)-Val-OH, 4 (0.343g, 0.66 mmol) was coupled using DCCI (0/15g, 0.73mmol) and HOBt (0.20g, 1.32 mmol) for 16 hours, then actylated for 1 hour.

After deprotection, Boc-His(DNP)-OH (1.26g, 3.0 mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6mmol) for 2 hours, then acetylated for 1 hour.

- 10 After deprotection, again with 50% TFA/CH₂Cl₂, Boc-Phe-OH (0.796g, 3mmol) was coupled with DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) for 3 hours then acetylated overnight.

After deprotection, Boc-Pro-OH (0.646g, 3mmol) was coupled using DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) for 2 hours then acetylated for 1 hour.

- 15 The peptide was again deprotected and coupled with Boc-His (DNP)-OH (1.26g, 3mmol) using DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) for 2 hours, then acetylated overnight.

The peptide resin ester was washed with DMF (3X), CH₂Cl₂ (3X) iPrOH (2X), CH₂Cl₂ (3X) and finally MeOH (3X) and dried. It was then treated with HF at 0° for 1½ hours in the presence of anisole (4ml) and dried overnight over potassium hydroxide. The resin was washed with DMF, acetic acid and acetic acid/water (1:1) to remove the peptide. The washes were combined and evaporated *in vacuo*.

- 20 The residue was dissolved in DMF (60 ml) and water (24ml), thioethanol (10ml) was added and the pH of the solution brought to 8.0 with sodium carbonate solution. The reaction mixture was stirred overnight, the solvent evaporated and the residue applied to a Sephadex G25 column (77 × 2.5cms). It was eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 34—53 were combined and the solvent evaporated *in vacuo* and dried.

25

Product C₄₉H₇₂O₃N₁₄ MW 985,21

Tlc (silica) R_f = 0.63 in EtOAc-Py-AcOH-H₂O (15:20:6:11)

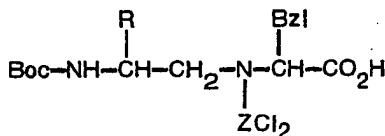
AA analysis in accordance with calculated composition.

30

Examples VI — IX

These Examples illustrate formula (VA). The methods disclosed above are applied to condensing Boc-Phe-H or Boc-Leu-H with H-Phe-OBzl, reducing the imine link, deprotecting at the carboxyl terminus and protecting the nitrogen of the reduced peptide link to give:

35



40

12 R = Bzl

12a R = iBu

ZCl₂ = 3,4-dichloro benzyloxy carbonyl

- 45 This Phe-reduced-Phe or Leu-reduced-Phe analogue is then used as follows:

- VI Use of 12 (Phe-reduced-Phe) in an analogue otherwise as H—77 (see Example IV)
- VII Use of 12a (Leu-reduced-Phe) in an analogue otherwise as H—77 (see Example IV)
- VIII Use of 12 in an analogue as H—76 (Example I), viz:

50

H-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH

(H 110)

6 7 8 9 10 11 12 13

55

- IX Use of 12a in an analogue as H—76 (Example I), viz:

H-His-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH

(H 115)

6 7 8 9 10 11 12 13

60

Example X

This Example illustrates formula (VB), the method of Example V being used but with the Tyr resin of Examples I to IV, to give:

65

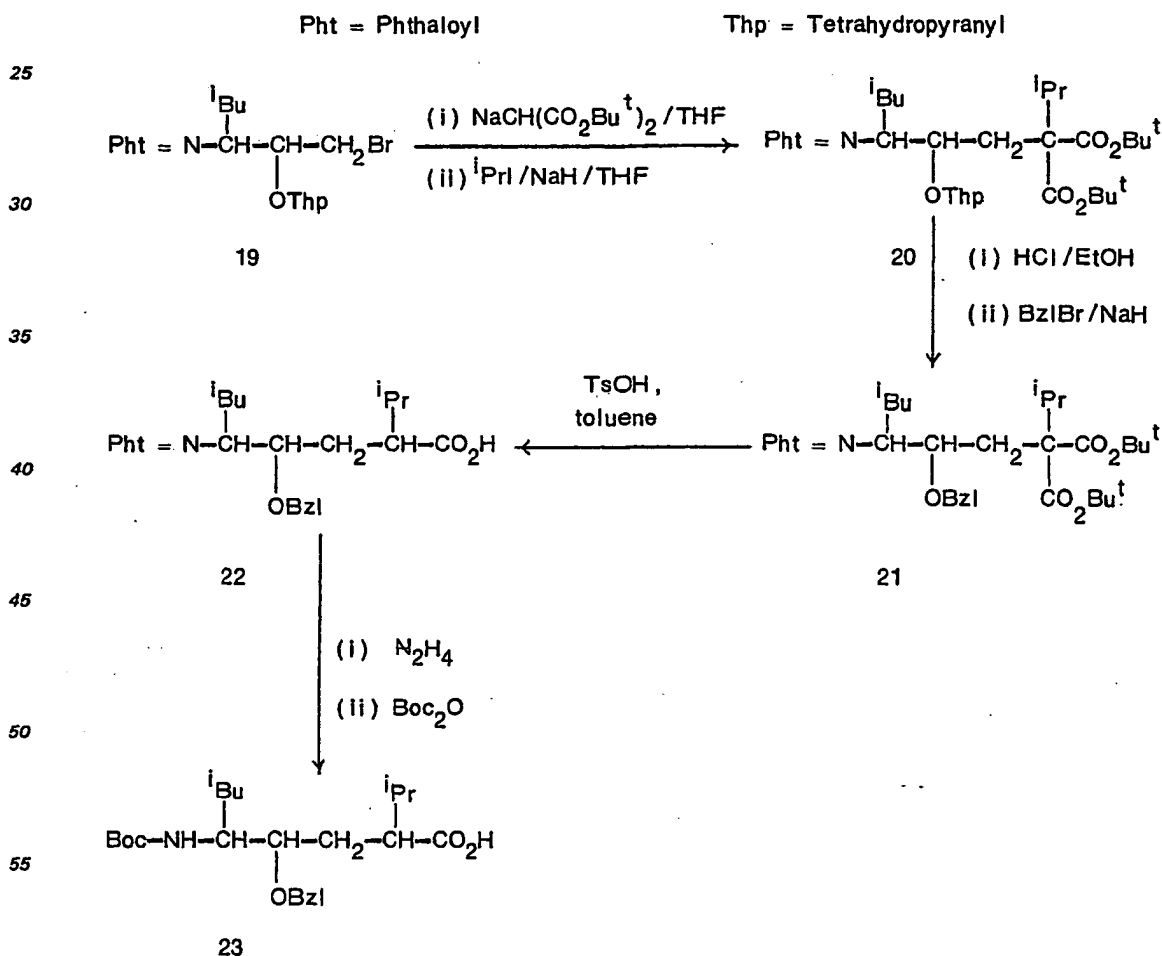
The resulting N-terminal phthaloyl protected, —OH protected hydroxy isostere of Leu-Leu can be coupled direct for example to valyl tyrosine, followed by removal of the phthaloyl group, coupling direct to a suitable tri or tetrapeptide, and deprotection at the —OH group by mild acid hydrolysis, to give for example analogues corresponding to H-76 (Example 1), H-79 (Example 2), H-77 (Example 4). Alternatively the phthaloyl group may be removed by treatment with hydrazine and a new protective group, e.g. benzyloxycarbonyl or t-butyloxycarbonyl attached prior to coupling. The methods used after the preparation of the protected hydroxy isosteres are those of the peptide synthesis art, well known in themselves and exemplified in detail herein. The compounds specifically prepared are:

- 10 a) H-His-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH
b) H-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH
c) H-DHis-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH

Example XIV

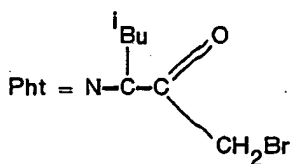
The alternative and preferred reaction scheme below, also suitable for other dipeptide hydroxy isosteres was used to synthesise an N-terminal and hydroxy-group protected Leu-Val hydroxy isostere 23.

SCHEME 5



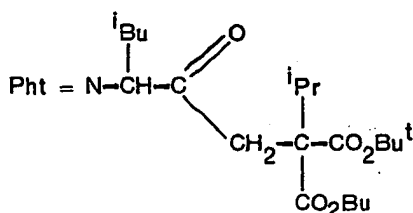
In the above scheme the protected bromohydrin **19** is obtained in the same way as the corresponding intermediate **17** in Scheme 4, and is subjected to malonic ester synthesis and alkylation with isopropyl iodide to give the malonic ester derivative **20**. Protection on the hydroxyl function is changed from Thp to Bzl to yield **21** and the latter is subjected to protonolysis and decarboxylation. In the resulting isostere acid

0 045. 665



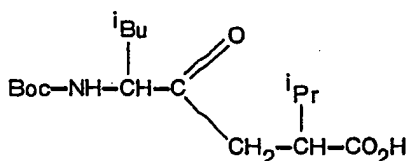
15

giving the compound



26

which is successively treated with TsOH, toluene and i) N_2H_4 , ii) Boc_2O to give:



25

ACTIVITY IN VITRO

Preliminary activity test results in the human renin-angiotensin substrate reaction in vitro are given in the table below, with comparative figures for the tetrapeptide analogue (III). The test is based on the methods described by J. A. Millar et al. in *Clinica Chimica Acta* (1980) 101 5—15 and K. Poulsen and J. Jorgensen in *J. Clin. Endocrinol. Metab.* (1974) 39 816.

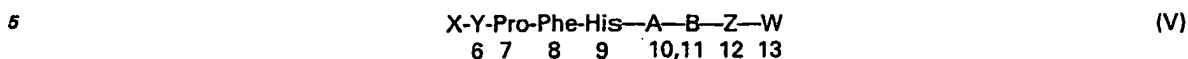
It is based on the measurement, by radioimmunoassay, of Angiotensin-I released from human renin substrate by human renin in human plasma. The inhibitor is dissolved in 0.01 N HCl (10 μl) containing EDTA, and angiotensin-I antibody (15 μl) in 3M-Tris/HCl buffer (pH 6.9).

After incubation at 37°C for 0—120 mins., the enzymic reaction is quenched by the addition of ice-cold 0.25M Tris/HCl buffer (pH 7.4) containing 0.01% of bovine serum albumin. ^{125}I -labelled angiotensin-I is added, followed by equilibration at 4°C for 48 hours. Free and bound ligand are separated by the addition of dextran-coated charcoal, and the amount of bound radio-ligand determined in a gamma counter.

The results for the renin inhibitory activities of the present compounds thus tested, expressed as the IC_{50} (the molar concentration required to cause 50% inhibition), are as follows:

Claims for the Contracting States: BE CH DE FR GB IT LI LU NL SE

1. A polypeptide analogue of the formula:

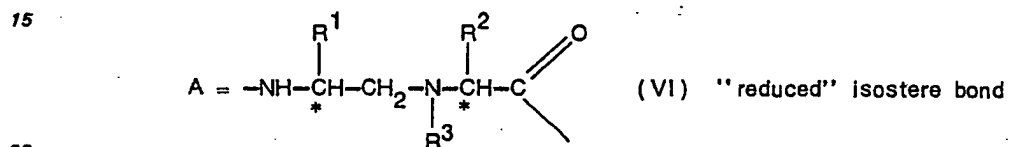


where:—

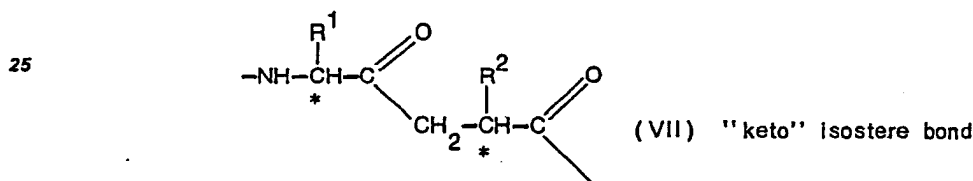
Pro, Phe and His may be in substituted form;

10 X = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, *t*-butoxycarbonyl (Boc), benzoyl or lower alkyl (primarily C₁—C₅); or an L- or D-amino- acyl residue, which may itself be N-protected similarly;

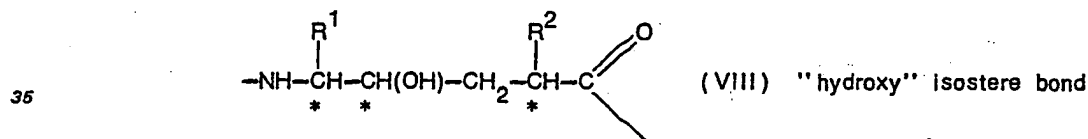
Y = D- or L-His or other D- or L- basic or aromatic amino-acyl residue, or is absent:



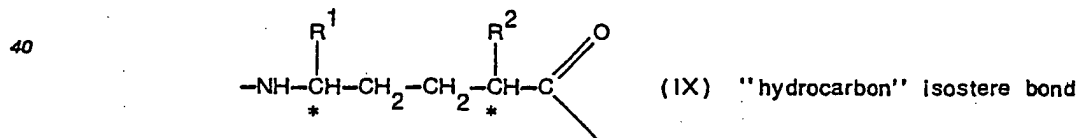
or



of



or



where the configuration at asymmetric centres * is either R or S, where in VIII the hydroxy group may be present as such or protected in ether —OR⁴ or ester



55 form where R^4 is as given under W below and where

R₁ and R₂, the same or different = 'Pro (isopropyl), 'Bu (isobutyl), Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain

$R^3 = -H$; lower alkyl (C_1-C_5); or $-SO_2Ph$, $-SO_2C_6H_4CH_3(p)$, Boc, formyl or other N-protecting group;

B = D- or L- Val or Ile or other D- or L- lipophilic amino-acyl residue;

60 Z = D- or L- Tyr, Phe, His or other L- or D-aromatic amino-acyl residue; and

W = —OH as such or in protected ester form as —OR⁴ where R⁴ = lower alkyl primarily C₁—C₅ and particularly 'Bu, or cycloalkyl primarily C₃—C₇, or Bzl, or other ester forming group; or —NH₂ as such or in protected amide form as —NHR⁵ or —N(R⁵)₂ (where R⁵ = an N-protecting or other substituent group e.g. lower alkyl as for R⁴ and (R⁵)₂ = two such or e.g. cycloalkyl, primarily C₃—C₇) or as —NH—(CH₂)_n—Q (where n = 2 to 6 and Q = NH₂ or

6. A polypeptide analogue as any one of claims 1 to 4 wherein the isosteric replacement at at least the 10,11 position is of the "hydroxy" kind.
7. The compound: H-His-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.
8. The compound: H-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.
9. The compound: H-His-Pro-Phe-His-Leu-reduced (SO₂Ph)-Leu-Val-Tyr-OH.
10. The compound: H-DHis-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.
11. The compound: H-His-Pro-Phe-His-Leu-reduced-Val-Ile-His-OH.
12. The compound: H-DHis-Pro-Phe-His-Phe-reduced-Val-Tyr-OH.
13. The compound: H-DHis-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH.
14. The compound: H-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH.
15. The compound: H-His-Pro-Phe-His-Leu-reduced-Val-Ile-Tyr-OH.
16. The compound: H-His-Pro-Phe-His-Leu-reduced-Val-Ile-Tyr-OH.
17. The compound: H-Pro-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-Lys-OH.
18. The compound: H-His-Pro-Phe-His-Leu-reduced-Val-Val-Tyr-OH.
19. The compound: H-His-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.
20. The compound: H-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.
21. The compound: H-DHis-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.
22. The compound: H-His-Pro-Phe-His-Leu-hydroxy-Val-Ile-His-OH.
23. The compound: H-His-Pro-Phe-His-Leu-keto-Val-Ile-His-OH.
24. A polypeptide analogue according to any one of claims 1 to 23, for use in a diagnostic test for high-renin states, blood pressure falling most when renin is high, or in a surgical prognostic test for renovascular hypertension (renal artery stenosis), by administration of said polypeptide analogue followed by monitoring of blood pressure.
25. A polypeptide analogue according to any one of claims 1 to 23, for use in the long and short term treatment of heart failure and all forms of hypertension particularly those associated with high serum renin levels, by administration of a renin-inhibiting amount of said polypeptide analogue.
26. A polypeptide analogue according to any one of claims 1 to 23, for use as a medicament, the dosage thereof being 0.001 to 10 mg/kg body weight daily, preferably 0.01 to 1.0 mg, of said polypeptide analogue.
27. A polypeptide analogue according to any one of claims 1 to 23, when in the form of a composition with a pharmaceutically acceptable diluent or carrier.
28. The composition of claim 27, in unit dosage form containing the amounts of analogue set out in claim 26 or sub-multiple thereof.

35 Claims for the Contracting State: AT

1. A process for preparing polypeptide analogues of the formula:

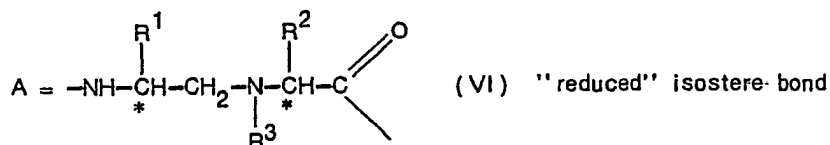


where:—

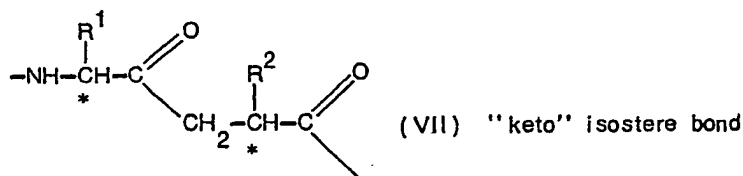
Pro, Phe and His may be in substituted form;

X = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, *t*-butoxycarbonyl (Boc), benzoyl or lower alkyl (primarily C₁—C₅); or an L- or D- amino- acyl residue, which may itself be N-protected similarly;

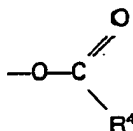
Y = D- or L-His or other D- or L- basic or aromatic amino-acyl residue, or is absent;



or



or



5

Form,

worin R^4 wie unter W unten angegeben ist, anwesend sein kann; und worin

10 R^1 und R^2 gleich oder verschieden sind und ^1Pro (Isopropyl), ^1Bu (Isobutyl), Bzl (Benzyl) oder eine andere lipophile oder aromatische Aminosäureseitenkette bedeuten;

R^3 für $-\text{H}$; Niedrigalkyl (C_1-C_3); oder $-\text{SO}_2\text{Ph}$, $-\text{SO}_2\text{C}_6\text{H}_4\text{CH}_3(\text{p})$, Boc, Formyl oder eine andere N-Schutzgruppe steht.

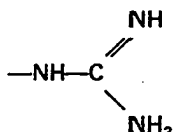
B für D- oder L- Val oder Ile oder einen anderen D- oder L-lipophilen Aminoacylrest steht;

Z für D- oder L- Tyr, Phe, His oder einen anderen L- oder D-aromatischen Aminoacylrest steht; und

15 W für $-\text{OH}$ als solches oder in geschützter Esterform als $-\text{OR}^4$, worin R^4 Niedrigalkyl, in erster Linie C_1-C_5 und insbesondere ^1Bu , oder Cycloalkyl, in erster Linie C_3-C_7 , oder Bzl, oder eine andere esterbildende Gruppe ist; oder $-\text{NH}_2$ als solches oder in geschützter Amidform als $-\text{NHR}^5$ oder $-\text{N}(\text{R}^5)_2$ (worin R^5 eine N-Schutzgruppe oder eine andere Substituentengruppe, z.B. Niedrigalkyl, wie für R^4 ist und $(\text{R}^5)_2$ = zwei solche, oder z.B. Cycloalkyl, in erster Linie C_3-C_7 darstellt) oder als $-\text{NH}-(\text{CH}_2)_n-\text{Q}$ oder

20

$-\text{NR}^5-(\text{CH}_2)_n-\text{Q}$ (worin n für 2 bis 6 und Q für NH_2 oder



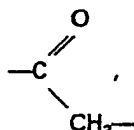
25

stehen und worin jeder der an Stickstoff gebundenen Wasserstoffe durch R^5 oder $(\text{R}^5)_2$ substituiert sein kann); ein L- oder D- Serin oder Lysin, Arginin oder einen anderen basischen Aminoacylrest als solchen

30 oder in Amidform, substituierter Amidform oder Esterform, z.B. eine Gruppe oder Gruppen enthaltend, wie für R^4 und R^5 oben fallweise angegeben; oder einen davon abgeleiteten Aminoalkoholrest als solchen oder geschützt in Ester- oder Ätherform, z.B. eine Gruppe enthaltend, wie für R^4 oben angegeben; steht oder

Z + W für einen Alkohol, abgeleitet von L- oder D- Tyr, Phe, His oder einem anderen L- oder D-aromatischen Aminoacylrest als solchen oder geschützt in Ester- oder Ätherform wie oben, stehen;

35 welches Polypeptid in obiger Form oder modifiziert durch isosteren Austausch einer oder mehrerer verbleibender Peptidbindungen durch reduzierte, $-\text{CH}_2-\text{NH}-$, Keto-,



40

Hydroxy-, $-\text{CH}(\text{OH})-\text{CH}_2-$ oder Kohlenwasserstoff-, $-\text{CH}_2-\text{CH}_2-$ isostere Bindeglieder vorliegt und

45 weiters in freier Form oder in geschützter Form an einer oder mehreren verbleibenden Peptid-, Carboxyl-, Amino-, Hydroxy- oder anderen reaktiven Gruppen vorliegt.

2. Polypeptidanalogue nach Anspruch 1 mit der Formel



(VA)

50

6 7 8 9 10,11 12 13

worin

X, Y, Pro, und His wie in Anspruch 1 definiert sind,

55 A wie in Anspruch 1 definiert ist, ausgenommen, daß R^1 und R^2 gleich oder verschieden sind und ^1Bu (Isobutyl) oder Bzl (Benzyl) oder eine andere lipophile oder aromatische Aminosäureseitenkette darstellen, R^3 für $-\text{H}$ oder $-\text{SO}_2\text{Ph}$, $-\text{SO}_2\text{C}_6\text{H}_4\text{CH}_3(\text{p})$, Boc, Formyl oder eine andere N-Schutzgruppe steht;

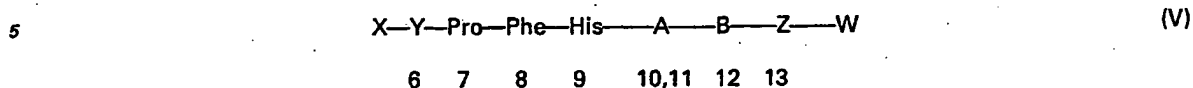
Z für Tyr, Phe oder einen anderen L- oder D- aromatischen Aminoacylrest steht;

60 W für $-\text{OH}$ als solches oder in geschützter Esterform als $-\text{OR}^4$, worin R^4 Niedrigalkyl (in erster Linie C_1-C_5 und insbesondere ^1Bu) oder Bzl, oder eine andere esterbildende Gruppe darstellt; oder für $-\text{NH}_2$ als solches oder in geschützter Amidform als $-\text{NHR}^5$ oder $-\text{N}(\text{R}^5)_2$ (R^5 ist eine N-Schutzgruppe, z.B. Niedrigalkyl wie für R^4 ; $(\text{R}^5)_2$ bedeutet zwei solche oder z.B. Cycloalkyl, in erster Linie C_3-C_7) oder für einen L- oder D- Aminoacylrest, z.B. einen Serin- oder basischen Aminoacylrest als solchen oder in Amidform oder in geschützter Amidform oder Esterform, z.B. eine Gruppe oder Gruppen enthaltend, wie sie für R^4

65 und R^5 oben fallweise angegeben sind; oder für einen davon abgeleiteten Aminosäurealkoholrest als

Patentansprüche für den Vertragsstaat: AT

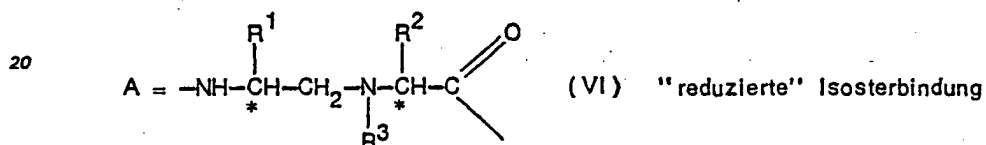
1. Verfahren zur Herstellung von Polypeptidanalogen mit der Formel:



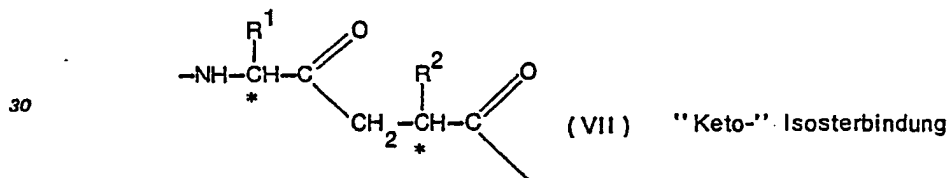
worin

10 Pro, Phe und His in substituierter Form vorhanden sein können;
X gleich H ist; oder ein Acyl oder eine andere N-Schutzgruppe, wie Acetyl, Pivaloyl, *t*-Butyloxycarbonyl (Boc), Benzoyl oder Niedrigalkyl (insbesondere C₁—C₅); oder ein L- oder D-Aminoacylrest, welcher selbst ähnlich N-geschützt sein kann, ist.

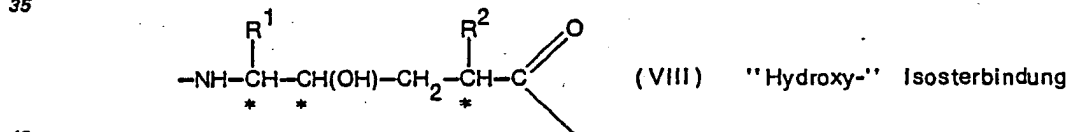
Y für D- oder L-His oder einen anderen D- oder L- basischen oder aromatischen Aminoacylrest steht,
15 oder abwesend ist:



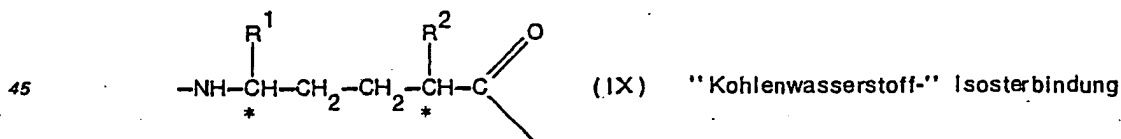
25 oder



oder



oder



⁵⁰ worin die Konfiguration an asymmetrischen Zentren* entweder R oder S ist, wobei in VIII die Hydroxygruppe als solche oder geschützt in Äther- —OR⁴ oder Ester-

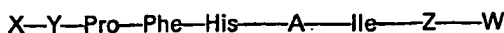


Form.

60 worin R⁴ wie unter W unten angegeben ist, anwesend sein kann; und worin
R¹ und R² gleich oder verschieden sind und 'Pro (Isopropyl), 'Bu (Isobutyl), Bzl (Benzyl) oder eine
andere lipophile oder aromatische Aminosäureseitenkette bedeuten;

R³ für —H; Niedrigalkyl (C₁—C₆); oder —SO₂Ph, —SO₂C₆H₄CH₃(p), Boc, Formyl oder eine andere N-Schutzgruppe steht.

65 B für D- oder L- Val oder Ile oder einen anderen D- oder L-lipophilen Aminoacylrest steht;



(VB)

6 7 8 9 10,11 12 13

5 worin

X, Y, Pro, Phe und His wie in Anspruch 1 definiert sind;

A wie in Anspruch 1 definiert ist, ausgenommen, daß R¹ für ¹Bu (Isobutyl) oder Bzl (Benzyl) oder eine andere lipophile oder aromatische Aminosäureseitenkette steht; R² für ¹Pr (Isopropyl) steht; und R³ für —H oder —SO₂Ph, —SO₂C₆H₄CH₃(p), Boc, Formyl oder eine andere N-Schutzgruppe steht;

10 Z wie in Anspruch 1 definiert ist;

W wie in Anspruch 2 definiert ist; oder

Z + W für einen Alkohol, abgeleitet von den in Anspruch 1 für Z spezifisch angeführten aromatischen Resten als solchen oder geschützt in Ester- oder Ätherform, wie hierin spezifiziert, stehen; welches Verfahren dadurch gekennzeichnet ist, daß die entsprechenden individuellen Peptidbaugruppen nacheinander ein zeln oder als präsynthetisierte Einheiten von zwei oder mehreren Gruppen zur Reaktion gebracht werden.

4. Verfahren zur Herstellung von Polypeptidanalogen nach einem der Ansprüche 1 bis 3, gekennzeichnet durch isosteren Austausch, wie hierin dargelegt, an einem oder beiden der Pro-Phe oder Phe-His Bindeglieder.

20 5. Verfahren zur Herstellung von Polypeptidanalogen nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß der isostere Austausch an mindestens der Position 10,11 der "reduzierten" Art entspricht.

6. Verfahren zur Herstellung von Polypeptidanalogen nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß der isostere Austausch an mindestens der Position 10,11 der "Hydroxy" Art entspricht.

25 7. Verfahren zur Herstellung der Verbindung H-His-Pro-Phe-His-Leu-reduziertes-Leu-Val-Tyr-OH oder H-Pro-Phe-His-Leu-reduziertes-Leu-Val-Tyr-OH oder

H-His-Pro-Phe-Leu-reduziertes(SO₂Ph)-Leu-Val-Tyr-OH oder

H-DHis-Pro-Phe-His-Leu-reduziertes-Leu-Val-Tyr-OH oder

30 H-His-Pro-Phe-His-Leu-reduziertes-Val-Ile-His-OH oder

H-DHis-Pro-Phe-His-Phe-reduziertes-Phe-Val-Tyr-OH oder

H-DHis-Pro-Phe-His-Leu-reduziertes-Phe-Val-Tyr-OH oder

H-His-Pro-Phe-His-Phe-reduziertes-Phe-Val-Tyr-OH oder

H-His-Pro-Phe-His-Leu-reduziertes-Phe-Val-Tyr-OH oder

35 H-His-Pro-Phe-His-Leu-reduziertes-Val-Ile-Tyr-OH oder

H-Pro-His-Pro-Phe-His-Phe-reduziertes-Phe-Val-Tyr-Lys-OH oder

H-His-Pro-Phe-His-Leu-reduziertes-Val-Val-Tyr-OH oder

H-His-Pro-Phe-His-Leu-Hydroxy-Leu-Val-Tyr-OH oder

H-Pro-Phe-His-Leu-Hydroxy-Leu-Val-Tyr-OH oder

40 H-DHis-Pro-Phe-His-Leu-Hydroxy-Leu-Val-Tyr-OH oder

H-His-Pro-Phe-His-Leu-Hydroxy-Val-Ile-His-OH oder

H-His-Pro-Phe-His-Leu-Keto-Val-Ile-His-OH,

welches Verfahren dadurch gekennzeichnet ist, daß die entsprechenden individuellen Peptidbaugruppen nacheinander einzeln oder als präsynthetisierte Einheiten von zwei oder mehreren Gruppen zur Reaktion gebracht werden.

Revendications pour les Etats contractants: BE CH DE FR GB IT LI LU NL SE

1. Homologue de polypeptide de formule:

50



(V)

6 7 8 9 10,11 12 13

55 dans laquelle:

Pro, Phe et His peuvent être sous forme substituée;

X représente H ou un groupe acyle ou autre groupe de protection de l'azote, par exemple un groupe acétyle, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl ou alkyle inférieur (principalement C₁—C₅), ou un reste L- ou D- aminoacyle, qui peut lui-même être protégé de la même façon sur l'azote;

60 Y représente D- ou L-His ou un autre reste D- ou L-aminoacyle basique ou aromatique, ou est absent;

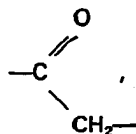
65

ou protégé sous forme d'ester ou d'éther, contenant par exemple un groupe tel qu'indiqué pour R⁴ ci-dessus, ou

Z + W représentent un alcool dérivé de L- ou D-Tyr, Phe, His ou d'un autre reste L- ou D-aminoacyle aromatique tel quel ou protégé sous forme d'ester ou d'éther comme ci-dessus;

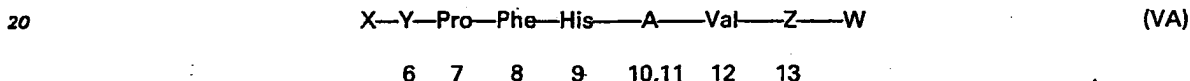
- 5 un tel polypeptide étant sous la forme ci-dessus ou étant modifié par remplacement isostérique d'une ou plusieurs des liaisons peptidiques restantes par les liaisons isostériques qui sont la liaison réduite, —CH₂—NH—, cétonique,

10



- 15 hydroxyle, —CH(OH)—CH₂—, ou hydrocarbonée, —CH₂—CH₂—, et étant de plus sous forme libre ou sous forme protégée sur un ou plusieurs des groupes restants peptides, carboxyles, amines, hydroxyles ou autres groupes réactifs.

2. Homologue de polypeptide selon la revendication 1, de la formule:

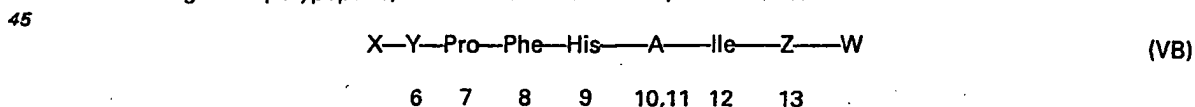


dans laquelle:

- 25 X, Y, Pro, Phe et His sont comme à la revendication 1
A est comme à la revendication 1, sauf que:
R¹ et R², identiques ou différents, représentent ^tBu (groupe isobutyle) ou Bzl (groupe benzyle) ou une autre chaîne latérale amino-acide lipophile ou aromatique,
R³ représente —H; ou —SO₂Ph, —SO₂C₆H₄CH₃(p), Boc, un groupe formyle ou un autre groupe de
30 protection de l'azote;
Z représente Tyr, Phe, ou un autre reste L- ou D- amino-acyle aromatique;
W représente —OH tel quel ou protégé sous forme d'ester —OR⁴, où R⁴ est un groupe alkyle inférieur, (principalement en C₁—C₅ et en particulier ^tBu), ou Bzl, ou un autre groupe donnant un ester; ou représente —NH₂ tel quel ou en donnant une forme amide protégée représente —NHR⁵ ou —N(R⁵)₂ (R⁵ représente un
35 groupe de protection de l'azote, par exemple un groupe alkyle inférieur comme pour R⁴; (R⁵)₂ représente deux groupes de ce genre ou, par exemple, un groupe cycloalkyle, principalement en C₃—C₇) ou représente un reste L- ou D- amino-acyle, par exemple un reste sérine ou amino-acyle basique tel quel ou sous forme amide ou sous forme amide protégé ou ester, contenant par exemple un ou des groupes tels qu'indiqués pour R⁴ et R⁵ ci-dessus selon le cas; ou représente un reste alcoolique, dérivé des amino-acides précédents,
40 tel quel ou protégé sous forme d'ester ou d'éther contenant par exemple un groupe tel qu'indiqué pour R⁴ ci-dessus, ou bien

Z + W représentent un alcool provenant de Tyr ou Phe ou d'un autre reste L- ou D- amino-acyle aromatique tel quel ou protégé sous forme d'ester ou d'éther comme ci-dessus.

3. Analogue de polypeptide, selon la revendication 1, de formule:

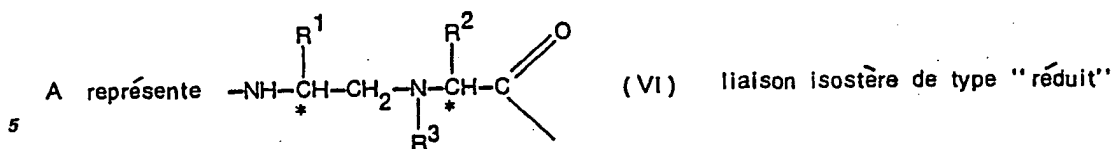


50 dans laquelle:

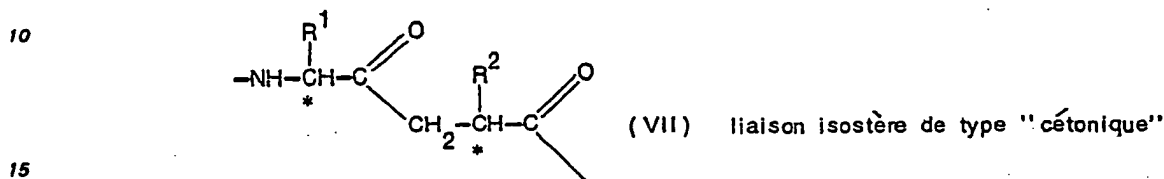
- X, Y, Pro, Phe et His sont comme à la revendication 1,
A est comme à la revendication 1, sauf que:
R¹ représente ^tBu (isobutyle) ou Bzl (benzyle) ou une autre chaîne latérale amino-acide lipophile ou aromatique,
55 R² représente ⁱPr (isopropyle), et
R³ représente —H, ou —SO₂Ph, —SO₂C₆H₄CH₃(p), Boc un groupe formyle ou un autre groupe de protection de l'azote,
Z est comme à la revendication 1,
W est comme à la revendication 2, ou
60 Z + W représentent un alcool dérivé des restes aromatiques spécifiés pour Z à la revendication 1, tel quel ou protégé sous forme d'ester ou d'éther, comme spécifié à la revendication 1.

4. Homologue de polypeptide selon l'une quelconque des revendications 1 à 3, modifié par remplacement isostérique, comme indiqué dans celles-ci, pour une des liaisons Pro-Phe ou Phe-His ou pour les deux.

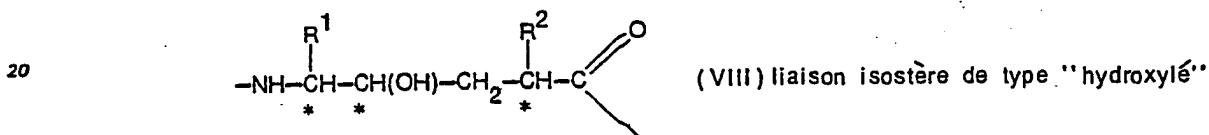
- 65 5. Homologue de polypeptide selon l'une quelconque des revendications 1 à 4, dans lequel il y a



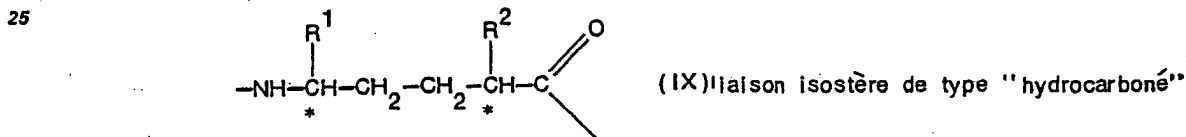
ou



ou



ou



25 de telle sorte que la configuration des centres asymétriques soit R ou S, et où dans VIII le groupe hydroxyle peut être présent tel que ou protégé sous forme d'éther —OR^4 ou d'ester



35 tel que R^4 soit comme indiqué en W ci-dessous, et où R^1 et R^2 identiques ou différents, représentent 'Pro (isopropyle), 'Bu (isobutyle), Bzl (benzyle) ou une autre chaîne latérale lipophile ou aromatique, R^3 représente —H ; un groupe alkyle inférieur ($\text{C}_1\text{—C}_5$); ou $\text{—SO}_2\text{Ph}$, $\text{—SO}_2\text{C}_6\text{H}_4\text{CH}_3(\text{p})$, Boc, formyle ou

40 un autre groupe de protection de l'azote;

B représente D- ou L- Val ou Ile ou un autre reste D- ou L- aminoacyle lipophile;

Z représente D- ou L- Tyr, Phe, His ou un autre reste L- ou D- aminoacyle aromatique; et

45 W représente —OH tel que ou protégé sous forme d'ester —OR^4 , où R^4 est un groupe alkyle inférieur, principalement en C_1 à C_5 et en particulier 'Bu, ou un groupe cycloalkyle, principalement en C_3 à C_7 , ou Bzl, ou un autre groupe formant un ester; ou représente —NH_2 tel que ou en donnant une forme amide protégée représente —NHR^5 ou $\text{—N(R}^5)_2$ (où R^5 est un groupe de protection de l'azote ou un autre substituant par exemple un groupe alkyle inférieur comme pour R^4 et $(\text{R}^5)_2$ représente deux groupes de ce genre ou, par exemple, un groupe cycloalkyle, principalement en C_3 à C_7) ou encore $\text{—NH—(CH}_2)_n\text{—Q}$ ou

50 $\text{—NR}^5\text{—(CH}_2)_n\text{—Q}$ (où n est 2 à 6 et Q représente NH_2 ou



60 avec n'importe lequel des atomes d'hydrogène fixés sur l'azote pouvant être substitué par R^5 ou $(\text{R}^5)_2$; ou représente un reste L- ou D- sérine ou lysine, arginine ou un autre reste aminoacyle basique tel quel ou sous forme d'amide, d'amide substitué ou d'ester, contenant par exemple un ou des groupes tel qu'indiqué

65 pour R^4 et R^5 ci-dessus selon le cas; ou représente un reste aminoalcool qui en dérive tel quel ou protégé

à 3, caractérisé en ce qu'on effectue un remplacement isostérique comme indiqué dans celles-ci, à l'une des liaisons Pro-Phe ou Phe-His ou aux deux.

5. Procédé de préparation d'homologues de polypeptides selon l'une quelconque des revendications 1 à 4, caractérisé en ce qu'on effectue un remplacement isostérique au moins à la position 10, 11 et de type "réduit".

6. Procédé de préparation d'homologues de polypeptides selon l'une quelconque des revendications 1 à 4, caractérisé en ce qu'on effectue un remplacement isostérique au moins à la position 10, 11 et du type "hydroxylé".

7. Procédé de préparation du composé: H-His-Pro-His-Leu-réduit-Leu-Val-Tyr-OH ou

10 H-Pro-Phe-His-Leu-réduit-Leu-Val-Tyr-OH ou
H-His-Pro-Phe-His-Leu-réduit (SO₂Ph)-Leu-Val-Tyr-OH ou

H-DHis-Pro-Phe-His-Leu-réduit-Leu-Val-Tyr-OH ou

H-His-Pro-Phe-His-Leu-réduit-Val-Ile-His-OH ou

H-DHis-Pro-Phe-His-Phe-réduite-Phe-Val-Tyr-OH ou

15 H-DHis-Pro-Phe-His-Leu-réduit-Phe-Val-Tyr-OH ou

H-His-Pro-Phe-His-Phe-réduit-Phe-Val-Tyr-OH ou

H-His-Pro-Phe-His-Leu-réduit-Phe-Val-Tyr-OH ou

H-His-Pro-Phe-His-Leu-réduit-Val-Ile-Tyr-OH ou

H-Pro-His-Pro-Phe-His-Phe-réduit-Phe-Val-Tyr-Lys-OH ou

20 H-His-Pro-Phe-His-Leu-réduit-Val-Val-Tyr-OH ou

H-His-Pro-Phe-His-Leu-hydroxylé-Leu-Val-Tyr-OH ou

H-Pro-Phe-His-Leu-hydroxylé-Leu-Val-Tyr-OH ou

H-DHis-Pro-Phe-His-Leu-hydroxylé-Leu-Val-Tyr-OH ou

H-His-Pro-Phe-His-Leu-hydroxylé-Val-Ile-His-OH ou

25 H-His-Pro-Phe-His-Leu-cétonique-Val-Ile-His-OH

ledit procédé étant caractérisé en ce que les groupes individuels correspondants constitutifs du peptide sont mis successivement à réagir seuls ou sous forme d'unités pré-obtenues de deux ou plusieurs groupes.

30

35

40

45

50

55

60

65